Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

A NEW MICROSCOPIC METHOD OF COUNTING BACTERIA ADAPTABLE TO ALL GRADES OF RAW AND PASTEURIZED MILK

PAUL W. ALLEN

From the University of Illinois, Urbana, Illinois.

One of the greatest difficulties in the way of the bacteriologic control of market milk is the lack of a suitable means of determining the number of bacteria per c.c. at all stages of production and handling. The plate method is not adaptable, as it requires a laboratory and at least a few days for incubation of the plates, during which time the milk is consumed before the count is known. The microscopic method of counting bacteria in which the $\frac{1}{100}$ c.c. pipet is used has little accuracy until the milk contains about 300,000 bacteria per c.c.

It is with the above facts in view that the writer has been carrying on during several years a somewhat continuous attempt to find a method of counting bacteria in milk when the number per c.c. ranges from 3000 to 300,000. The following method has been found successful in so far as it has been used as is shown by the results here reported. However it is realized that a final verdict can be given only after several laboratories report favorable results.

THE METHOD

The method is based on the fact that a water suspension of aluminum hydroxid readily collects the bacteria in milk and that the centrifuge is able to throw down the aluminum hydroxid containing the bacteria to one end of the centrifuge tube leaving the fat, casein, and water very largely behind. The aluminum hydroxid is readily transferred to a glass slide and dried down. As aluminum hydroxid shrinks to a very considerable degree on drying a thin microscopic film is made possible. In staining the films it is found that the hydroxid has slight affinity for methylene blue and that on washing them with water a clear field is obtained in which the bacteria stand out by contrast.

THE METHOD IN DETAIL

Preparation of the Hydroxid Suspension.—Mix equal parts of N/20 $AlK(SO_4)_2.12H_2O$ and N/20 $NaOH$. After precipitation is complete wash the precipitate thoroughly by decantation using scrupu-

Received for publication December 3, 1917.

lously pure distilled water, keeping in mind that any dirt in the wash water will be taken up by the aluminum hydroxid and will appear in the film under the microscope. Make up to original volume.

Procedure.—1. Add to the sample of milk to be analyzed enough of the washed aluminum hydroxid precipitate suspension so that it becomes 20% of the mixture. Shake thoroughly for several minutes.

2. Add 2.5 c.c. of the above mixture to a centrifuge tube holding this amount when stoppered. (Use plain centrifuge tube open at both ends for stoppers.)

3. Centrifuge for 15 minutes at 5000 revolutions per minute.

4. Pull stopper at cream end of tube and remove the cream with a needle allowing the milk to run out. Then carefully pull the stopper at the other end of the tube and transfer the plug of aluminum hydroxid to a clean glass slide laying over glass or cardboard ruled off in sq. cm. By use of a needle or loop grind up the precipitate on the slide and spread it evenly over an area of 2 sq. cm. using a loopful of sterile water if necessary for uniform spreading.

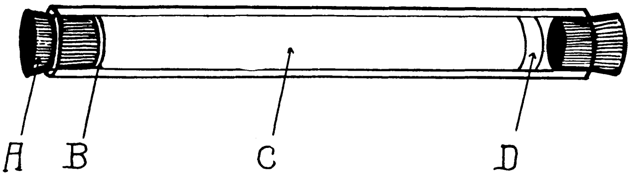

Fig. 1.—Centrifuge tube. Length of the tube is 8 cm.; inside diameter 8 mm. *A*, rubber stopper; *B*, plug of $Al_2(OH)_6$; *C*, milk free from fat and $Al_2(OH)_6$; *D*, butter fat.

5. Place in incubator until nearly dry, then finish drying at room temperature.

6. Heat slightly several inches above Bunsen flame.

7. Place in tray of toluene and agitate tray several minutes to remove butter fat, than take slides out of tray and allow the toluene to evaporate from them.

8. Place slides in 95% alcohol to remove all traces of toluene.

9. Stain in 25% saturated aqueous solution of methylene blue until the film reaches a proper depth.

10. Dry in the warm air above a Bunsen flame and examine under the microscope, using an oil immersion lens of which the number of fields per sq. cm. has been determined.

TABLE 1

COMPARISON OF THE HYDROXID METHOD OF COUNTING BACTERIA IN MILK WITH THE PLATE METHOD AND THE BREED METHOD

Sample of Milk, Number	Kind of Milk	Plate Method Count	Hydroxid Method Count		Breed Method Count	
		Bacteria per C.c.	Bacteria per Average Field	Bacteria per C.c.	Bacteria per Average Field	Bacteria per C.c.
101	Raw	92,500	35.2	105,600	0.5	150,000
102	Raw	114,000	52.0	156,000		
103	Raw	103,600	60.9	182,700	0.3	100,000
104	Raw	326,600	119.4	358,200	2.3	690,000
105	Raw	68,000	25.7	77,000	0.4	120,000
106	Raw	353,300	141.5	424,500	1.5	450,000
107	Raw	263,300	123.6	370,800		
108	Raw	170,000	108.6	325,800	1.0	300,000
109	Raw	88,000	61.8	155,400	0.5	150,000
110	Raw	108,000	55.3	165,900	0.9	270,000
111	Raw	82,000	40.8	122,000		
112	Raw	16,600	7.6	22,800		
113	Raw	87,000	47.2	141,600	0.7	210,000
114	Pasteurized	8,060	5.5	16,500		
115	Pasteurized	6,630	3.1	9,300		
116	Raw	106,000	36.4	104,200	0.7	210,000
117	Raw	294,600	143.2	429,600	1.2	460,000
118	Raw	230,000	99.6	298,800	1.6	480,000
119	Raw	73,300	23.3	69,900	0.1	30,000
120	Raw	142,300	74.5	223,500	1.7	510,000
121	Raw	266,300	156.4	469,200	2.1	630,000
122	Raw	126,000	63.5	180,500	0.6	180,000
123	Raw	311,600	118.0	354,000	0.7	210,000
124	Raw	666,000	267.7	803,100	1.4	420,000
125	Raw	5,430,000	Thousands	....	27.4	8,220,000
126	Raw	4,160,000	Thousands	....	29.2	8,760,000
127	Raw	466,000	205.9	617,700	2.8	840,000
128	Raw	936,000	296.1	888,300	3.7	1,110,000
129	Raw	3,200,000	Thousands	....	19.0	5,715,000
130	Raw	30,600	14.9	44,700	0.2	60,000
131	Raw	3,083	1.8	5,400		
132	Raw	930,000	307.6	922,800	3.5	1,065,000
133	Raw	1,700,000	720.0	2,157,000	7.6	2,295,000
134	Raw	923,300	470.0	1,410,600	4.5	1,365,000
135	Raw	676,000	365,0	1,095,000	5.0	1,500,000
136	Raw	846,000	319.4	958,200	3.6	1,080,000
137	Raw	1,253,000	570.0	1,710,000	7.1	2,130,000
138	Raw	690,000	367.6	1,102,800	5.2	1,560,000
139	Raw	44,600	21.7	65,100	1.3	390,000
140	Raw	135,300	71.4	214,200	1.1	330,000
141	Raw	1,466,000	Thousands	....	30.2	9,060,000
142	Raw	11,033,000	Thousands	....	72.8	21,840,000
143	Raw	18,930	7.9	23,700		
144	Raw	1,100,000	517.6	1,552,000	6.0	1,600,000
145	Pasteurized	13,500	4.5	13,500		
146	Pasteurized	7,400	3.0	9,000		
147	Pasteurized	8,230	3.1	9,300		
148	Pasteurized	10,800	4.5	13,500		
149	Pasteurized	14,400	5.1	15,300		
150	Pasteurized	7,640	3.3	9,900		
151	Pasteurized	14,800	5.3	15,900		
152	Pasteurized	9,166	4.5	13,500		
153	Pasteurized	28,060	12.6	37,800		
154	Pasteurized	1,376	0.5	1,500		
155	Pasteurized	2,216	0.9	2,700		
156	Pasteurized	9,930	4.3	12,900		
157	Pasteurized	130,000	57.0	161,000		
158	Pasteurized	12,160	4.4	13,200		
159	Pasteurized	7,100	4.0	12,000		
160	Pasteurized	9,433	3.2	9,600		
161	Pasteurized	9,430	5.2	15,600		

TABLE 1—Continued

COMPARISON OF THE HYDROXID METHOD OF COUNTING BACTERIA IN MILK WITH THE PLATE METHOD AND THE BREED METHOD

Sample of Milk, Number	Kind of Milk	Plate Method Count	Hydroxid Method Count		Breed Method Count	
		Bacteria per C.c.	Bacteria per Average Field	Bacteria per C.c.	Bacteria per Average Field	Bacteria per C.c.
162	Pasteurized	5,000	2.0	6,000		
163	Pasteurized	16,660	5.6	16,800		
164	Pasteurized	68,600	33.2	996,000	0.4	120,000
165	Pasteurized	3,730	1.0	3,000		
166	Pasteurized	1,660	0.6	1,800		
167	Pasteurized	37,700	2.4	72,000		
168	Pasteurized	5,050	1.8	5,400		
169	Pasteurized	700	0.8	2,400		
170	Pasteurized	3,513	2.1	6,300		
171	Pasteurized	13,560	4.9	14,700		
172	Pasteurized	280	0.2	600		
173	Pasteurized	5,700	3.2	4,800		
174	Pasteurized	3,360	3.8	5,700		
175	Pasteurized	8,500	6.6	9,900		
176	Pasteurized	5,860	2.5	7,500	0.1	30,000
177	Raw	97,300	40.6	121,800		
178	Pasteurized	4,420	1.9	5,700		
179	Pasteurized	7,630	2.9	7,700		
180	Pasteurized	20,000	16.9	25,000		
181	Pasteurized	4,700	2.3	6,900		
182	Pasteurized	3,860	1.5	4,500		
183	Pasteurized	14,100	3.2	9,600		
184	Pasteurized	13,200	7.3	21,900		
185	Pasteurized	6,730	2.3	6,900		
186	Pasteurized	18,730	6.8	20,400		
187	Pasteurized	6,900	2.3	7,800		
188	Pasteurized	28,330	15.5	46,500		
189	Pasteurized	47,000	16.8	50,400	0.8	240,000
190	Pasteurized	1,816	0.8	2,400		
191	Pasteurized	10,566	4.5	13,500	0.1	30,000
192	Pasteurized	10,660	5.5	16,500	0.1	30,000
193	Pasteurized	18,300	6.3	18,900	4.4	120,000
194	Pasteurized	3,800	1.9	4,800	0.1	30,000
195	Raw	2,046,000	Thousands	....	12,5	3,750,000
196	Raw	2,110,000	Thousands	....	15.5	4,650,000
197	Raw	410,000	170.1	541,280	3.6	1,146,240
198	Raw	916,000	385.8	1,225,840	5.8	1,783,000
199	Raw	1,250,000	457.0	1,371,000	5.8	1,783,000
200	Raw	670,000	282.0	897,000	7.5	2,260,600
201	Raw	733,000	306.0	919,000	4.7	1,410,000

11. Count 20 representative fields on the 2 sq. cm. and obtain the average number of bacteria per field.

12. The number of bacteria per c.c. of milk is obtained by multiplying the average number of bacteria per field by the number of microscopic fields in a sq. cm. Usually there are a little over 3000 fields per sq. cm. when the ordinary 1.8 oil immersion lens is used, but using 3000 as an even number to multiply by is satisfactory in counting.

PRECAUTIONS

If so much aluminum hydroxid precipitate is thrown down in the centrifuge that it makes too thick a film when spread over 2 sq. cm. the hydroxid suspension should be diluted. The thickness of the plug of aluminum hydroxid after centrifugalization should be about 1 mm.

TABLE 2

DETERMINATION OF THE EFFICIENCY OF ALUMINUM HYDROXID IN REMOVING THE BACTERIA FROM MILK

Milk Sample Number	Kind of Milk	Milk before Mixing with $Al_2(OH)_6$ and Centrifuging Bacteria per C.c.	Milk and Cream from Centrifuge Tube Mixed after Centrifuging Out the $Al_2(OH)_6$; Bacteria per C.c.	Percentage of Bacteria Removed by $Al_2(OH)_6$ and Centrifugalization
1	Pasteurized	1,220	46	96.3
2	Pasteurized	2,660	17	99.4
3	Pasteurized	20,500	210	98.8
4	Pasteurized	12,900	180	98.6
5	Pasteurized	14,900	390	97.4
6	Pasteurized	62,000	2,200	96.5
7	Raw	51,000	1,620	96.9
8	Raw	34,100	2,300	93.3
9	Raw	111,000	4,500	96.0
10	Raw	16,300	170	99.0
11	Raw	4,560	390	91.5
12	Raw	3,900	460	88.3
13	Raw	6,120	110	93.3
14	Raw	4,320	280	93.7
15	Raw	2,640	270	89.8
16	Raw	6,100	440	92.8
17	Raw	6,840	610	91.1
18	Raw	5,280	420	92.1
19	Raw	68,300	5,760	91.6
20	Raw	2,110,000	66,000	96.9
21	Raw	540,000	27,000	95.0
22	Raw	724,000	22,000	97.0
23	Raw	18,200	610	96.7
24	Raw	70,300	7,600	89.2
25	Raw	88,400	1,270	98.6
26	Raw	14,200	980	93.1
27	Raw	26,600	1,570	94.1
28	Raw	44,000	6,300	98.6
29	Pasteurized	2,740	310	88.7
30	Pasteurized	5,280	425	92.0
31	Raw	72,400	6,180	91.5
32	Pasteurized	3,460	40	98.9
33	Pasteurized	6,120	110	98.3
34	Pasteurized	49,000	2,400	95.2
35	Pasteurized	6,540	670	89.8
36	Pasteurized	1,730	86	95.1
37	Raw	44,400	310	99.3
38	Raw	13,200	140	99.0
39	Raw	63,000	5,100	92.0
40	Raw	1,185,000	69,000	94.3
41	Raw	3,170,000	264,000	91.9

No fat or toluene should appear in the film under the microscope. Some samples of pasteurized milk show fine granular material or little curd particles due to heating. This may be removed by filtering through a small amount of cotton.

The film should stain uniformly all over. The best depth is sky blue. Too deep a stain can be moderated by dipping the film in alcohol.

Rubber stoppers must be used with centrifuge tubes, as cork will not hold the liquid in the centrifuge.

The temperature of the milk during centrifugalization should be from 25-30 C.

TABLE 3

THE DETERMINATION OF THE ACCURACY OF THE HYDROXID METHOD OF COUNTING BACTERIA BY A REPETITION OF COUNTS ON THE SAME SAMPLES OF MILK

Sample of Milk, Number	Kind of Milk	Number of Count on Same Sample of Milk	Bacteria per Field	Bacteria per C.c.
I	Pasteurized	1	8.2	25,600
		2	8.8	25,400
		3	8.7	26,100
		4	8.4	25,200
		5	9.0	27,000
		6	8.2	24,600
		7	10.6	31,800
		8	8.6	25,800
		9	10.4	31,200
		10	8.3	24,900
		11	8.0	24,000
		12	10.0	30,000
		13	10.1	30,300
		14	8.3	24,900
		15	10.5	31,500
		16	10.8	32,400
		17	9.6	28,800
		18	9.4	28,200
		19	8.0	24,000
		20	8.4	25,200
II	Raw	1	35.3	105,900
		2	34.4	103,200
		3	32.7	98,100
		4	36.3	108,900
		5	33.9	101,700
		6	35.4	106,200
		7	30.8	92,400
		8	34.9	104,700
		9	35.4	106,200
		10	32.9	98,700
		11	34.8	104,400
		12	35.9	107,700
		13	35.2	105,600
		14	37.6	112,800
		15	34.7	104,100
		16	35.2	105,600
		17	34.7	104,100
		18	36.7	110,100
		19	36.7	110,100
		20	39.9	110,700

Some practice in the technic is necessary before the best results are obtained.

COMPARISON OF THE HYDROXID METHOD OF COUNTING BACTERIA IN MILK WITH THE PLATE METHOD AND THE BREED METHOD

Data Concerning Table 1.—The samples of milk used were stirred thoroughly, then immediately plated which required about 10 minutes for the making of the dilutions and the pouring of the agar. Then smears were made according to the hydroxid method and according to the 1/100 c.c. pipet method (Breed method).

In plating standard 1% lactose agar was used. Incubation of plates was 2 days at 37 C. and 3 days at 20 C. Triplicate plates in 3 dilutions were made of each sample. The decimal system of dilution was used.

In counting the bacteria in the hydroxid method and in the Breed method 20 representative fields were counted on each smear and the average number of bacteria per field used.

DETERMINATION OF THE EFFICIENCY OF ALUMINUM HYDROXID IN REMOVING THE BACTERIA FROM MILK

Data Concerning Table 2.—Samples of milk were plated before being mixed with aluminum hydroxid or centrifuging. Then after mixing the milk with the hydroxid suspension, shaking thoroughly, and centrifuging for 15 minutes the milk and cream were poured from the centrifuge tube into 8 c.c. of sterile water, shaken thoroughly and plated using media, and incubation time and temperature as in Table 1.

CONCLUSIONS

From the foregoing data concerning the hydroxid method of counting the bacteria in milk it is concluded that about 95% of the bacteria in the average sample of milk appear in the hydroxid thrown down by centrifugalization.